Studies on Lipoarabinomannan as a Rapid Diagnostic Marker for Tuberculosis

A

SYNOPSIS

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Introduction

Tuberculosis (TB) is an airborne disease caused by *Mycobacterium tuberculosis*. The *Mycobacterium tuberculosis (*MTB), the causative agent of tuberculosis disease was discovered by Robert Koch in 1882 **(Koch, 1982)**. Later on, TB has been characterized as an airborne disease that generally affects the lungs with the characteristics like severe coughing, chest pain, loss of appetite and fever etc. At the time of discovery, the ratio of TB affected person was as high as that one out of every seven people has been died because of TB in Europe and United States. The reason behind its severity is fine droplets produced during pulmonary TB through sneezing, coughing or speaking and these droplets spread disease from an infected person to a healthy person by inhalation process **(Riley and Moodie, 1974)**.The size of droplet nuclei up to 5μm and contain as few as 1-3 bacilli. The largest droplet falls on the ground but a smaller size droplet can remain airborne for a prolonged period of time and eventually inhaled by another person **(Yates *et al.*, 2016)**. Pulmonary TB based infection is characterized as two types i.e. latent TB and active TB. Most infected individuals are asymptomatic, which means that they have latent TB and maintain the infection in a quiescent form with no clinical symptoms evidence of disease **(Comstock *et al.*, 1974)**. Epidemiological studies of both developing and developed countries indicate that 5–10% of latent individuals will develop active TB during their lifespan **(Vynnycky and Fine, 2000)**. Active TB is the condition of an infected person in which the immune system of that person unable to find off or defend against the *Mycobacterium tuberculosis.* Active TB is a fatal disease. TB is a disease of destitution and poverty **(Spence *et al*., 1993)** as lacking of basic health services, social disruption, malnutrition, and inadequate and scanty living conditions contribute to the dissemination of TB and its impact in the community. HIV infection and Acquired Immune Deficiency Syndrome (AIDS) are the strongest and firm risk factor for TB **(Millet *et al*., 2013)**.

WHO declared tuberculosis as a global public health emergency in 1993 ended a period of prolonged global neglect. TB is among the top ten causes of global mortality **(Murray and Lopez, 1997)**. It is estimated that approximately one-third of the world population is infected with tuberculosis bacillus, and each year eight million people develop tuberculosis disease which annually kills 1.8 million

worldwide **(WHO, 2001)**. TB is the second most fatal disease after AIDS as 9 million cases were reported in 2014 with 16.67% mortality and 3.5% MDR-TB cases **(WHO report, 2014)**.In 2014, **"post- 2015 global TB strategy"** was announced by the World Health Assembly, to end the global TB epidemic with targets to reduce TB deaths by 95% and reduce TB incident cases by 90% until 2035 **(WHO, 2015)**. Globally in 2017, there were an estimated 10 million incident cases of TB (range, 9.0–11.1 million), equivalent to 133 cases (range, 120–148) per 100,000 population **(WHO, 2017)**.

As per the global TB report of 2017, the estimated incidence of TB in India was around 2.74 million accounting for about a quarter of the TB cases globally **(WHO, 2017)**. As per the **"Post-2015global TB strategy"** India has set the target for complete elimination of TB incidences and death by 2025, ten years ahead of the global target of 2035. "Swachh Bharat Abhiyan" is supposedly also playing a key role to achieve this goal.

Mycobacterium tuberculosis is a pathogenic bacteria member of the *Mycobacteriaceae* family and the causative agent of tuberculosis. The organisms were named so because of the mold like (myco: fungus; bacterium: bacteria) pellicular growth of these organisms in a liquid medium **(Collins *et al.*, 2014)**. *Mycobacterium tuberculosis* is an exceptionally successful obligate pathogen with unique characteristic features which make it highly pathogenic **(Brennanand Nikaido,1995)**.*Mycobacterium tuberculosis* was first revealed by Robert Koch in 1882. This finding by Robert Koch brought him the honour of the Nobel Prize in 1905.*Mycobacterium tuberculosis* genome size is 4 million base pairs which contain ~4000 genes, of which 40% are characterized, 44% have probable functions, while the remaining 16% are uncharacterized **(Cole *et al.*, 1998)**.The guanine and cytosine (G+C) ratio in the DNA of *Mycobacterium* is 62 to 70 mol percent **(Runyon, 1959)**.*Mycobacterium tuberculosis* has an unusual waxy coating on its cell surface due to the presence of mycolic acid. This coating makes the cells impervious to gram staining thus acid-fast stains such as Ziehl-Neelsen or fluorescent stains such as auramine are used to identify *Mycobacterium tuberculosis* by microscopy **(Fu and Fu-Liu, 2002)**.*Mycobacterium tuberculosis* is categorized as acid-fast and gram-positive due to the presence of thick peptidoglycan**(Brennan, 2003)**. *Mycobacterium tuberculosis* divides in ~18-20 hour and

resides in the human alveolar macrophages. TB infection may occur either by activation of already existing latent bacilli or through new bacilli. Generally, equilibrium exists between active and latent bacilli and disruption of this equilibrium causes acute infection. According to the dynamic re-infection hypothesis, non-replicating bacteria will drain constantly towards the bronchial tree and spread infection, which is once again followed by the generation of granuloma **(Cardona, 2009)**. The cell wall of *Mycobacterium tuberculosis* is composed of 60% of lipids. Major fraction of its cell wall is mycolic acid, cord factor, and wax-D **(Alderwick *et al.*, 2007)**.The cell wall of *Mycobacterium tuberculosis* is composed of two segments, the outer part, and the core of the cell wall. Core of cell wall is made up of peptidoglycan (PG), covalently attached with arabinogalactan (AG) and mycolic acids subsequently, forming the mycolylarabinogalactan peptidoglycan (mAGP) complex **(Hett and Rubin, 2008)**. The upper part is composed of free lipids and contains different cell wall proteins, the phosphatidylinositol mannosides (PIMs), Lipomannan (LM), and Lipoarabinomannan (LAM) **(Rao and Meena, 2011)**. There are so many antigens present in *Mycobacterium tuberculosis* and some major antigens are - Total mycolic acid-containing glycolipid (TBGL), cord factor, sulpholipid I and lipoarabinomannan (LAM).

Lipoarabinomannan (LAM), the major antigenic glycolipid of *Mycobacterium tuberculosis*, is an important immunodiagnostic target for detecting tuberculosis (TB) infection **(Choudhary *et al.*, 2018)**. LAM is a major structural component of the *Mycobacterium tuberculosis* cell wall and an important mediator of functions that promote productive infection and pathogenicity **(Kallenius *et al.*, 2016)**.Antigenic molecules in the LAM consist of repeating five linked D-arabinofuranose residues. The epitopes on this molecule are located on the surface of the cell wall in *M. tuberculosis*, whereas in *M.leprae* they have an inside orientation. LAM is water-soluble, protease-resistant and resistant to boiling, is supposed to be degraded slower than protein molecules because of its polysaccharide nature **(Patil *et al.*, 1995)**. LAM is a glycoconjugate and one of the virulence factors associated with *Mycobacterium tuberculosis*. It is a major cell wall component and allows the *Mycobacterium tuberculosis* to survive in the host cell environment by affecting host resistance and immune responses **(Guerardel *et al.*, 2003)**.LAM can account for up to 15% of the bacterial total weight.

LAM of *Mycobacterium tuberculosis* has 17.3kDa molecular weight according to several MALDI-MS studies **(Venisse *et al.*, 1993)**.LAM is mainly made up of three components: a phosphatidylinositol membrane anchor, a (1→6)–linked mannan backbone of mannopyranose (Manp), and an arabinan chain containing multiple arabinofuranoside (Araf) residues with and hexa-Araf termini. Membrane anchor attaches the molecule to the cell wall at the time of infection, and homopolysaccharides serve as carbohydrate skeleton **(Nigou *et al.*, 2003)**. LAM is classified into three major classes based on the presence and structure of capping. Mannosylated LAMs (Man LAM), in which mannosyl groups are present on the D-arabinan group. After the mannosyl capping, Man LAM acts an anti-inflammatory molecule and inhibits the production of TNF-α and IL-12. These properties of Man LAM allow the bacteria to survive in the host cell for a long time. Man LAMs are mainly found in pathogenic mycobacterial species such as *M. tuberculosis*, *M. leprae*, and *M. bovis*. Phosphoinositol-capped LAM (PILAM) are LAMs capped with phosphoinositol groups and are mainly found in nonpathogenic species such as *M. smegmatis* **(Gilleron *et al.*, 1997)**. Arabinofuranosyl-Terminated LAM (Ara LAM), Ara LAM 1 and 3-mannosyl side chains are present in many mycobacterial species. Many other lipid components of bacterial cell wall like Lipomannan (LM) and phosphatidylinositol mannosides (PIMs) are involved in the synthesis of LAM. The LAM is synthesized by the addition of mannopyranosyl to a phosphoinositol. PIMs are considered as precursors of LAMs in the biosynthesis pathway **(Cotran *et al.*, 1989)**. PIMs and LM are synthesized by the addition of mannopyranosyl to a phosphoinositol. Glycosylation of PIMs and LM with arabinan forms LAM **(Besra*et al.*, 1997)**. Mannosyl transferases are involved in the synthesis of PIMs.

A substantial amount of clinical experience indicates that host immunity plays an important role in the host-pathogen interaction occurring in persons exposed to *M. tuberculosis* **(Schluger and Rom, 1998)**.The route of entry of the tubercle bacillus into the body is *via* the respiratory tract through the inhalation of respiratory droplet nuclei **(Riley *et al.*, 1959)**. Once organisms have made their way into the lung, they have many potential fates; (a) The initial host response can be completely effective and kill all the bacilli, (b) The organism may begin to multiply and grow immediately after infection-causing clinical disease known as primary tuberculosis, (c) the bacilli may become dormant and never

cause disease at all such that the patient has latent infection, and (d) the latent organisms can eventually begin to grow with resultant clinical disease, known as reactivation tuberculosis **(Dannenberg, 1994)**.Pathogenic mycobacteria use LAM, lipomannan (LM), glycolipid and glycoproteins surface-exposed molecules, for attachment, enter, and to infect host cells. These surface molecules specifically bind certain surface receptors on the host cell, alveolar macrophages **(Torrelles and Schlesinger, 2010)**.The process of phagocytosis is the first critical step for the infection of macrophages by mycobacteria. Mechanisms used by *Mycobacterium tuberculosis* to evade immune system of host cells are (a) phagosome maturation process is blocked, and (b) manipulation of intraphagosomal pH (**Gordon *et al.*, 1980)**, (c) in manipulation of host cytoskeleton system, mycobacteria can inhibit the antigen processing and presentation besides phagosome maturation and can further down-regulate the production of MHC class II molecules **(Noss *et al.*, 2001)**.LAM is an important cell wall component, and one of the most important virulence factors of *Mycobacterium tuberculosis*. These all mechanisms include the inhibition of the activity of macrophage protein kinase-C (PKC) and mitogen-activated protein kinase (MAPK) leading to the arrest of INF-γ transcription and the block of respiratory burst leading to neutralization of cytotoxic oxygen free radicals produced by macrophages **(Chatterjee and Khoo, 1998)**.

LAM inhibits T-cell proliferation and bactericidal activities of macrophages(**Cotran *et al.*,1989)**. LAM has the ability to bind with the Toll-like receptors (TLRs) and thus can insert itself into biological membranes affecting signaling events **(Brennan, 2003)**. ManLAM has been shown to have immunosuppressive effects by stimulating IL-10 production and it inhibits the production of IL-12 interfering with Toll-like receptors (TLRs) and tumornacrosis factor (TNF) production. ManLAM also modulates *Mycobacterium tuberculosis* induced macrophage apoptosis by binding with host mannose receptors and this is particularly important in deactivating host macrophages to allow the bacteria to survive and multiply within them **(Saleh *et al.*, 2016)**.

It is quite evident that the prevalence of TB disease is there since antiquity and it is notoriously fatal and contagious disease. Our study is towards one step to contribute global crusade to eradicate TB from the world and especially in our country. The major objective of this study is towards early detection of TB, which will be economical, rapid, and fast. Currently, there are lots of methods and

techniques for the diagnosis of TB, but all the methods are not very specific and sensitive for diagnosis of TB. With the help of this ongoing study based on immunological test (ELISA), we wish to diagnose TB in an early stage. In the proposed research work, we will focus to explore all the possible dimensions of immunogenic characteristics of LAM and standardize their antigenic properties for using LAM as a diagnostic marker. Henceforth, the ongoing study shall be very helpful towards the eradication of the TB from the world.

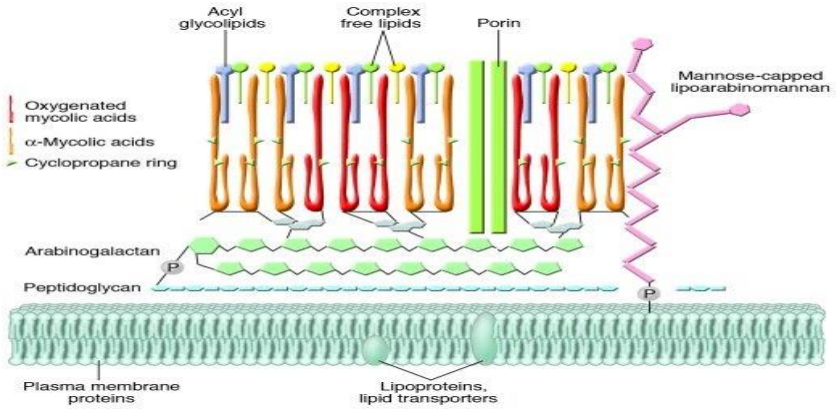


Figure 1: ***Mycobacterium tuberculosis*** **cell wall envelope (**Citation Information: *J Clin Invest.* 2006;116(6):1475-1478. https://doi.org/10.1172/JCI28734).

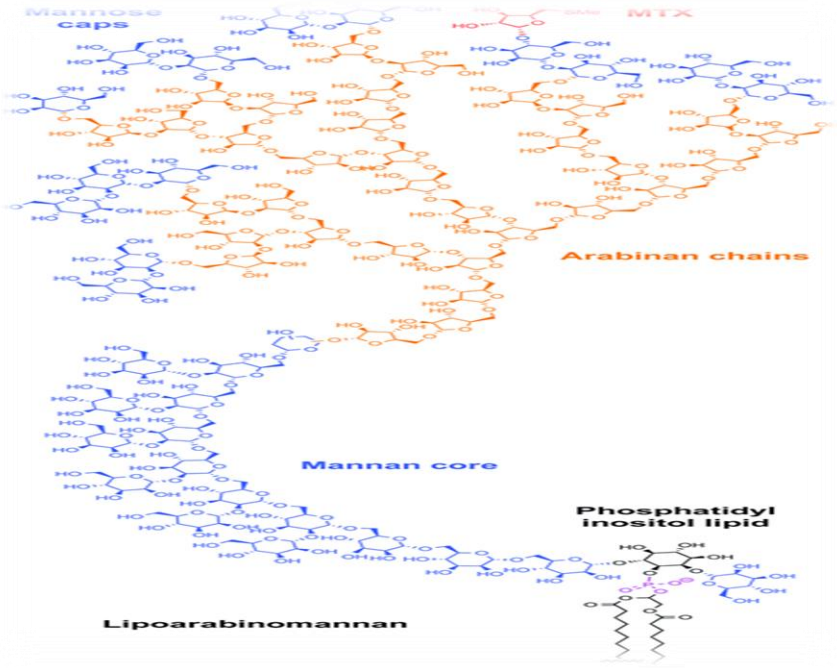


Figure 2: Structure of Mannose capped LAM in ***Mycobacterium tuberculosis*** Organic & Biomolecular Chemistry **Methylthioxylose – a jewel in the mycobacterial crown).**

Review of literature

To understand the previous work done by different researchers, we have studied the relevant literature, so that we can have a glance over the work already been done and what to be done further accordingly.

Chan *et al*., (1991) studied that Lipoarabinomannan as a possible virulence factor involved in the sustenance of *Mycobacterium tuberculosis* within macrophages and cause tuberculosis. Their results suggest that LAM can inhibit macrophage activation, triggering a cytocidal activity. **Besra *et al.*, (1997)** investigated the biosynthesis of *Mycobacterium* lipoarabinomannan (LAM) and they reported that mycobacterial lipoglycans, lipomannan (LM) and lipoarabinomannan (LAM) are potent immune modulators in tuberculosis. **Parrish *et al.*, (1998)** investigated the mechanism of latency in *Mycobacterium tuberculosis* that can persist within the human host for years without causing disease known as latent tuberculosis (TB). **Chatterjee and Khoo (1998)** studied the Mycobacterial lipoarabinomannan as an extraordinary lipoheteroglycan with profound physiological effects. **Arias-Bouda *et al.*, (2000)** investigated the development of antigen detection ELISA assay for diagnosis of tuberculosis using sputum samples and they concluded that thirty-one (91%) of 34 sputum samples from tuberculosis patients were positive in the LAM detection assay. In contrast, none of the 25 sputum samples from 21 non-tuberculosis patients was positive**. Chan *et al*., (2000)** investigated the diagnosis of tuberculosis by a visually detectable immunoassay for LAM. They have developed a simplified, visually detectable, colloidal gold-based serological assay to qualitatively detect IgG directed against the mycobacterial cell wall component LAM. They concluded that anti-LAM IgG immunoassay is relatively sensitive and specific for active TB and thus, a potentially useful screening test for active TB. **Hamasur *et al.*, (2001)** studied the rapid diagnosis of tuberculosis by detection of mycobacterial LAM in urine and they had used ELISA and dipstick test. They concluded that both methods were highly sensitive, detecting LAM at concentrations of 1.0 ng/ml and 5.0 pg/ml and 15 patients with active TB showed intermediate to high levels of LAM in their urine. **Khatri and Frieden (2002)** studied the controlling tuberculosis in India. They analyzed the effects of new policies introduced in 1993. **Movahedzadeh *et al*., (2004)** studied that the *Mycobacterium*

*tuberculosis*inositol-1-phosphate synthase (ino1) gene is essential for growth and virulence and they told that *Mycobacterium tuberculosis* utilizes inositol in the production of its major thiol and of essential cell wall lipoglycans. **Boehme *et al.*, (2005)** studied the detection of mycobacterial LAM with an antigen-capture ELISA in unprocessed urine of Tanzanian patients with suspected tuberculosis. They concluded that in Tanzania, 231 patients with suspected pulmonary TB and 103 healthy volunteers were screened with standard TB tests and with the new LAM-ELISA kit. Out of 132 patients with confirmed pulmonary TB (positive sputum culture), 106 were positive using the LAM-ELISA (sensitivity 80.3%). **Kang *et al.*, (2005)** studied that the human macrophage mannose receptor directs *Mycobacterium tuberculosis* lipoarabinomannan ManLAM mannose caps mediated phagosome biogenesis. *Mycobacterium tuberculosis* survives in macrophages in part by limiting phagosome– lysosome fusion. **Kaur *et al.*, (2008)** studied the mannose capping of lipoarabinomannan (LAM) by a multifunctional terminal mannosyltransferase. **Pitarque *et al.*, (2008)** studied the lipoglycans, lipoarabinomannan, lipomannan and all immunomodulatory molecules that are exposed at the mycobacterial cell surface. **Minion *et al.*, (2011)** evaluated the diagnosing of active tuberculosis with urine LAM by using urinary LAM assays. It is a potential marker of active tuberculosis. **Gounder *et al.*, (2011)** studied the diagnostic accuracy of urine LAM-ELISA for screening ambulatory HIV infected persons for TB. **Peter *et al.*, (2012)** studied the urine LAM strip-test diagnostic accuracy for TB detection in HIV-infected hospitalized patients. Their study indicates that the LAM strip test may be potentially useful for rapid rule-in test for TB in hospitalized patients with advanced immunosuppression. **Fukuda *et al.*, (2013)** studied the deletion or over-expression of mannosyl transferase enzyme results in gross changes in LM/LAM structures. They showed that such changes in LM/LAM structures have a significant impact on the cell wall integrity of mycobacteria and pathogenesis of tuberculosis. **Sarkar *et al.*, (2014)** studied the applications of lipoarabinomannan antigen in the detection of tuberculosis by using LAM assays. E**l-Atty*et al.*, (2014)** evaluated the value of serum lipoarabinomannan in the diagnosis of pulmonary tuberculosis. The serum LAM-ELISA test showed high positivity in confirmed TB patients and sputum smear combined with LAM diagnosed 95% of the confirmed cases. **Lawn and Gupta-Wright (2016)** investigated the detection of LAM in urine as an indicator of disseminated TB with renal involvement in patients living with

advanced immunodeficiency and HIV. Kidneys serve as the primary mechanism by which LAM enters the urine**. Caulfield and Wengenack (2016)** studied the diagnosis of active tuberculosis disease by using various methods such as acid-fast staining, culture of *Mycobacteria* and molecular technologies using definitive species identification such as nucleic acid hybridization probe, MALDI-TOF MS and Nucleic Acid Amplification Test (NAAT). **Saleh *et al.,* (2016)** studied the macrophage infection by *Mycobacterium tuberculosis.* Invasion of the macrophages protects the pathogen from attack by the immune system, allows it to multiply while protected within the macrophages, and alters the immune response. **Choudhary *et al.,* (2018)** studied the characterization of the antigenic heterogeneity of Lipoarabinomannan, the major surface glycolipid of *Mycobacterium tuberculosis*, and complexity of antibody specificities toward this antigen. **Amin *et al.,* (2018)** studied the detection of LAM in urine and serum of HIV-positive and HIV-negative TB suspects. They applied two strategies for the sensitive diagnosis of TB infection i.e. by using an improved capture-ELISA and by Gas Chromatography/Mass Spectrometry. **Correia- Neves *et al.,* (2018)** studied the usefulness of mycobacterial lipoarabinomannan (LAM) as a diagnostic marker for active and latent TB. **Kawasaki *et al.,* (2019)** evaluated the ability of immunoassay to measure the concentration of LAM in sputum to detect bacterial load and treatment response in patients with pulmonary tuberculosis. They isolated monoclonal antibodies for LAM by using phage display technology and develop a sandwich-ELISA (LAM-ELISA) to quantitate LAM concentration by using these monoclonal antibodies. **Younis *et al.,* (2019)** studied the combining serum antibody with urine lipoarabinomannan (U-LAM) detection as simple non-sputum based screening method for HIV-associated tuberculosis and they evaluated that combining U-LAM with IgG detection significantly increased the sensitivity for HIV-associated TB to 92% compared to 30% for U-LAM alone.

Objectives

1. To study Lipoarabinomannan (LAM) in sputum, as early and prolonged diagnostic marker of tuberculosis using LAM specific monoclonal antibody.
2. Comparative study of LAM in sputum and non-sputum (other body fluids like urine and serum) samples.
3. Testing of nasal specimens for the presence of LAM and acid-fast bacilli.
4. Testing of the above specimens in contacts of patients and healthy controls for the significance of these tests in disease diagnosis**.**

Material and methodology

Site of Sample Collection:

Diseased subjects samples for our study will be collected from JALMA Institute and/or S.N. Medical College, TB demonstration centre, Agra. The healthy subject samples will be collected from healthy laboratory volunteers working in the JALMA Institute, DEI University, and other volunteer organizations.

Subject size:

The proposed study shall be conducted with two cohorts of control and diseased group.

The diseased subjects of tuberculosis shall consist of 200 in number and an equal number of contacts and healthy controls (200 diseased+ 200 controls+ 200 contacts of patients = 600).

There is no age criterion for sample collection.

Inclusion and exclusion criteria of subjects-

- **Inclusion criteria of subjects:**

  Coughing that lasts two or more weeks, coughing up blood (hemoptysis), chest pain, painwith breathing (dyspnea) or coughing, unintentional weight loss, fatigue, fever, nightsweats, and positive culture test.

- **Exclusion criteria of subjects:**

The subjects co-suffering from HIV-AIDS, alcohol addicts and subjects with other known bacterial infections shall be excluded from the study.

Collection of sample (sputum, urine, blood, and nasal swab) with Consent form:

Consent form shall be filled-up and get signed, followed by collection of sputum, urine, blood and nasal swab shall be obtained in falcon tubes, sterile plastic containers and plain red cap vials respectively and stored at -20^{0}C until use.

Handling of samples:

- **Pretreatment of the sputum samples**-

  An equal volume of freshly prepared N-acetyl-L-cysteine-sodium hydroxide solution (NALC-NaOH) shall be added in the sputum sample. After 15 min of incubation, phosphate-buffered saline (pH 6.8) shall be added to make the volume to 50 mL. The mixture shall be centrifuged for 15 min at 3,000×g. The resultant supernatant will be discarded and the pellet shall be resuspended in a final volume of 2.5 mL phosphate-buffered saline (pH 6.8), of which 0.2 mL shall be used for LJ culture and TB detection **(Kawasaki *et al*., 2019)**.

- **Pretreatment of urine sample** -

  50 ml of urine sample would be sterilized by boiling at 100^{0}C for 10 min in a water bath and centrifuged at 4,000xg for 5 min at room temperature. The supernatant after centrifugation shall be concentrated to 5.0 ml and then diluted with PBS to 100 ml and concentrated again to 1.0 ml yielding a concentration of the original urine samples by 50 fold. The processed samples shall be then analyzed immediately or stored at -20^{0}C until analyzed **(Hamasur *et al.*, 2001)**.

- **Pretreatment of serum sample** -

  After collection of blood, allow the blood to clot by leaving it undisturbed in a slanting position at room temperature for almost 30 minutes. Serum shall be isolated from blood by centrifugation at 1,000-2,000xg at 4^{0}C for 10 min **(Youkhana *et al.*, 2015)**.The resulting supernatant is designated serum. The serum sample might be used immediately or shall be stored at –20^{0}C for further use.

Extraction of LAM for measurement:

To unmask the LAM antigen maximally from bacilli for detection, so many methods of extraction have been evaluated, resulting in the adoption of the following method for LAM extraction. Adding of 1.2 M NaOH solution to 0.4 ml of the sample (bacterial cell culture or sputum and urine), heating the mixture at 100˚C for 20 min, followed by adding 0.09 mL of 5 M of NaH_2PO_4. These LAM extracts shall be then stored at −70˚C until measured by LAM-ELISA **(Kawasaki *et al*., 2019)**.

Monoclonal antibody of LAM:

The monoclonal antibody against LAM being used for the proposed research study was developed by Prof. J. Ivanji of Wellcome Trust Laboratories, U.K., in collaboration with National JALMA Institute of Leprosy and Other Mycobacterial Diseases in 1981 (NJIL & OMD Indian Council of Medical Research, ICMR), Agra. The antibody is a kind gift of our co-supervisor Dr.Shripad A. Patil, Director, NJIL & OMD, Agra.

LAM measurement by the sandwich-ELISA:

LAM measurement will be conducted by adding monoclonal LAM antibody into the microtitre plate wells after dilution in carbonate buffer (pH 9.6) and incubated overnight at 4^0C. After washing the plate 4 times with PBS buffer then blocked plate with BSA or casein for 1 hour at 37ºC (**Mukundan *et al*., 2012)**. Sample of subjects will be placed in duplicates on ELISA plates and then incubated for 1 hour. After washing by PBS buffer, add HRP-conjugated LAM specific antibody then incubate for 1 hour at 37^0 C. Plate will be washed with PBS then add 0.1 ml of TMB substrate. After 10 min of incubation time, the substrate reaction will be stopped by adding 0.1 ml of H_2SO_4. Then colour development shall be read at 450 nm on a molecular devices absorbance plate or ELISA reader **(Boehme *et al.*, 2005)**.

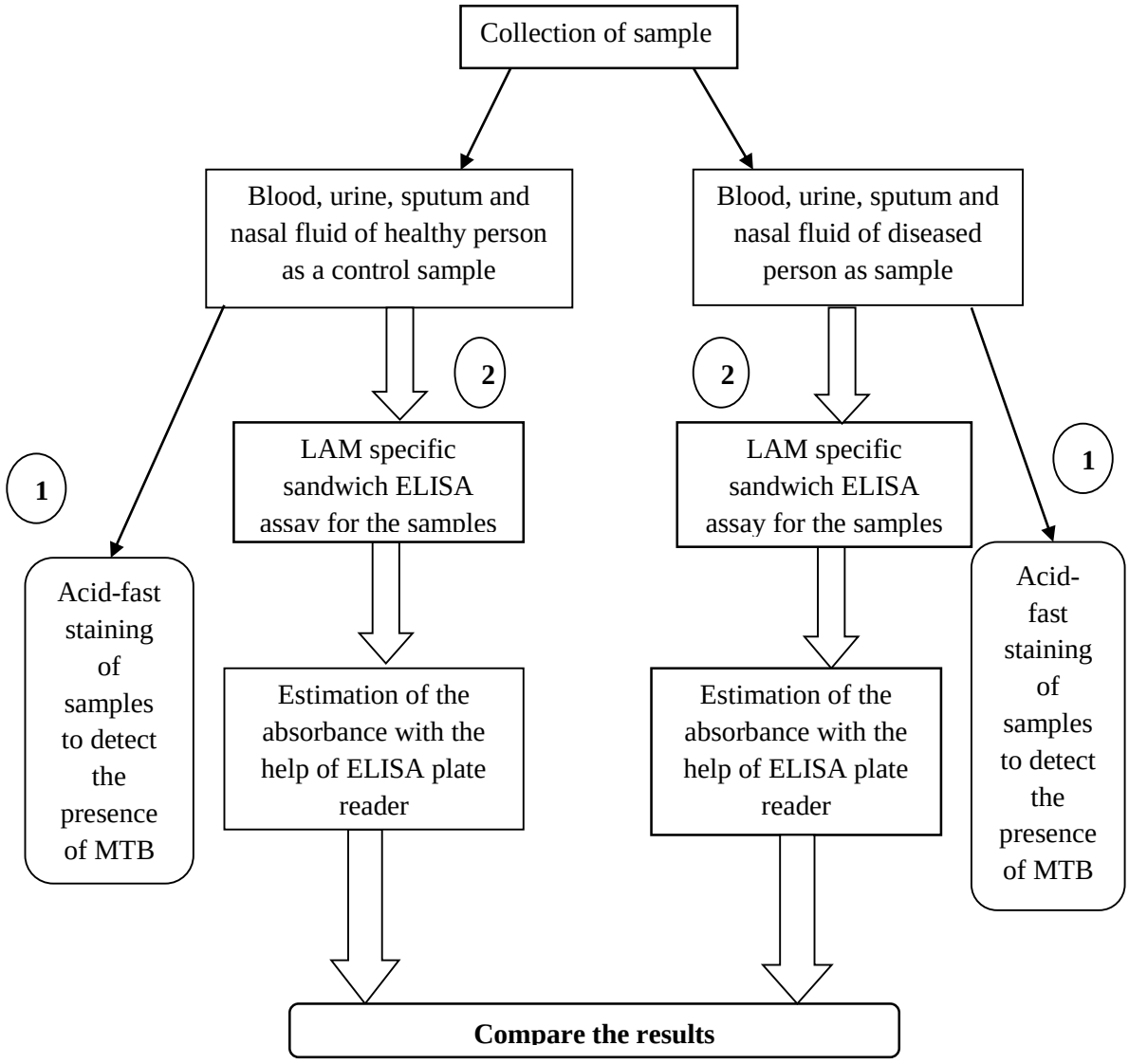
SDS-PAGE:

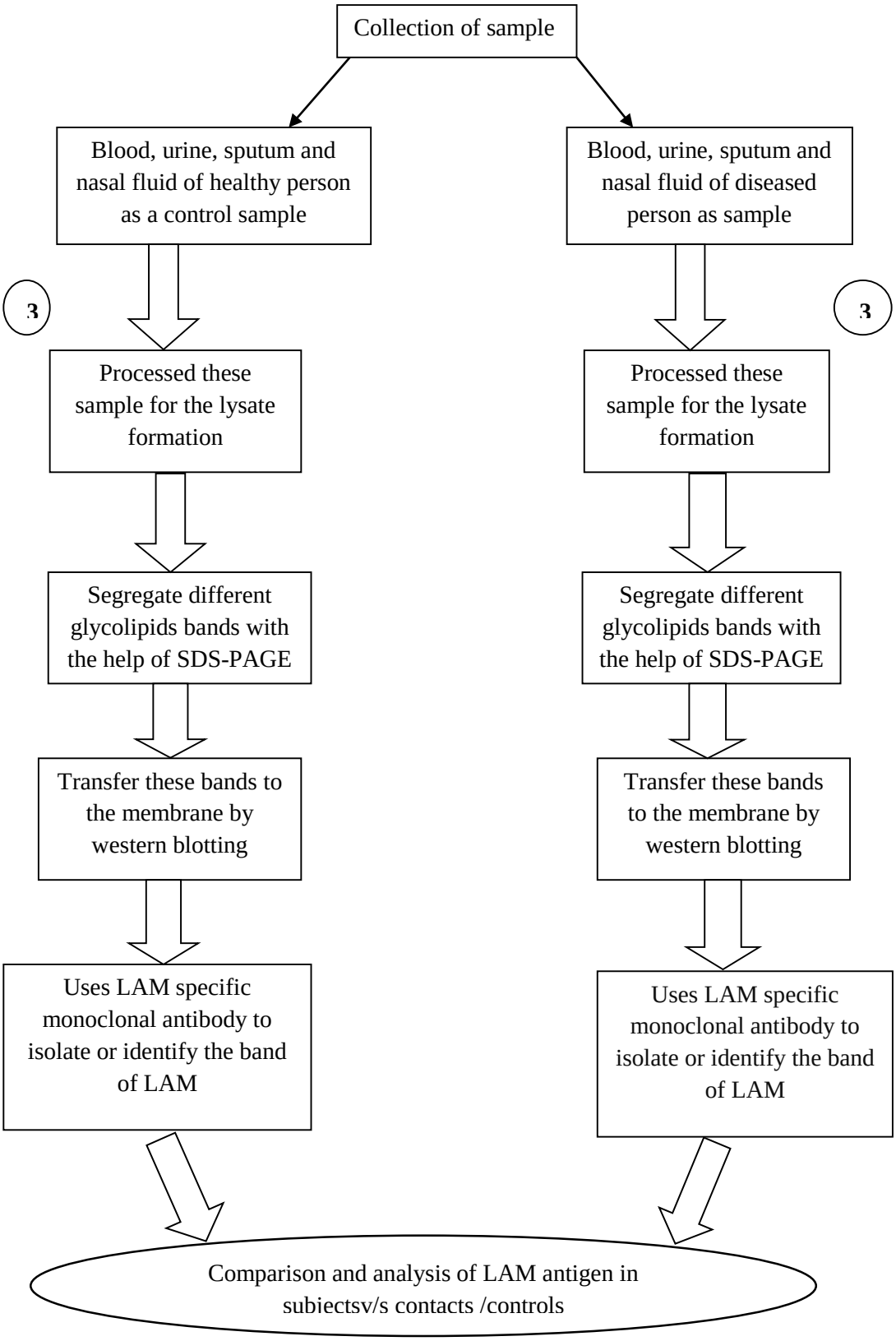
The LAM antigen will be examined by running the respective samples on SDS-PAGE for protein/LAM profile and to determine the molecular weight of the major protein/LAM band. After running the standards and the unknown sample, the gel is processed with the desired stain and then de-stained for about 12 to 14 hours to visualize the protein/LAM bands **(Hamasur *et al*., 1999)**.

Western blot:

Following SDS-PAGE run, western blotting will be done. Western blotting is the antibody-based detection and commonly used to detect proteins/LAM from samples. Bands of the sample will be moved from within the gel onto a membrane of nitrocellulose (NC) or polyvinylidenedifluoride (PVDF). The primary antibody specific to a target protein is incubated with the membrane and the primary antibody binds to target protein on the membrane. After rinsing the membrane to remove unbound primary antibody, the membrane is exposed to specific enzyme-conjugated secondary antibody. A substrate reacts with the enzyme (HRP) that is bound to the secondary antibody to generate colored substance to visualize protein bands **(Cruz Walma and Collins, 2019)**

Work plan


Collection of sample
Blood, urine, sputum and nasal fluid of healthy person as a control sample
Blood, urine, sputum and nasal fluid of diseased person as sample
2
2
1
1
LAM specific sandwich ELISA assay for the samples
LAM specific sandwich ELISA assay for the samples
Acid-fast staining of samples to detect the presence of MTB
Acid-fast staining of samples to detect the presence of MTB
Estimation of the absorbance with the help of ELISA plate reader
Estimation of the absorbance with the help of ELISA plate reader
Compare the results


Collection of sample
Blood, urine, sputum and nasal fluid of healthy person as a control sample
Blood, urine, sputum and nasal fluid of diseased person as sample
3
3
Processed these sample for the lysate formation
Processed these sample for the lysate formation
Segregate different glycolipids bands with the help of SDS-PAGE
Segregate different glycolipids bands with the help of SDS-PAGE
Transfer these bands to the membrane by western blotting
Transfer these bands to the membrane by western blotting
Uses LAM specific monoclonal antibody to isolate or identify the band of LAM
Uses LAM specific monoclonal antibody to isolate or identify the band of LAM
Comparison and analysis of LAM antigen in subiectsv/s contacts /controls

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